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$0.35 Estimated cost File1
$0.35 Estimated cost this search
$0.35 Estimated total session cost 0.101 DialUnits

File 155:MEDLINE(R) 1966-2003/Mar W1
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S2 46011 TERMINUS OR TERMINI
S3 141 S1 AND S2
S4 27843 TRANSPOS?
S5 1 S3 AND S4
S6 342515 LEFT OR RIGHT
S7 22 S3 AND S6
S8 9668 TELOMER?
S9 8 S1 AND S8 NOT S3
S10 74 BOVINE(W)PARVO?
? t s5/7/1
5/7/1
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.
04584837 84282662 PMID: 6088052
Rescue of adeno-associated virus from recombinant plasmids: gene correction within the terminal repeats of AAV.
Samulski R J; Srivastava A; Berns K I; Muzyczka N
Cell (UNITED STATES) May 1983, 33 (1) p135-43, ISSN 0092-8674
Journal Code: 0413066
Contract/Grant No.: 5 R01 AI16326; AI; NIAID; 5 T32 AI07110; AI; NIAID
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We have isolated three types of pBR322-AAV recombinant plasmids that contain deletions within the 145 bp AAV terminal repeats. When the plasmids were transfected into human cells, mutants that contained deletions within the left (type I) or right (type II) terminal repeat were viable. Of four mutants examined that contained deletions in both termini (type III), only one was viable. All of the viable mutants produced AAV virions that contained wild-type AAV DNA. Furthermore, the viable type III deletion could be converted to a nonviable mutant by deleting all copies of an 11 bp sequence from its termini. We conclude that there is an efficient mechanism for correcting deletions within the AAV termini. A model that could account for these observations is also discussed.
Record Date Created: 19840928
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5/5/1
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.
04584837 84282662 PMID: 6088052
Rescue of adeno-associated virus from recombinant plasmids: gene correction within the terminal repeats of AAV.
Samulski R J; Srivastava A; Berns K I; Muzyczka N
Cell (UNITED STATES) May 1983, 33 (1) p135-43, ISSN 0092-8674
Journal Code: 0413066
Contract/Grant No.: 5 R01 AI16326; AI; NIAID; 5 T32 AI07110; AI; NIAID
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS
We have isolated three types of pBR322-AAV recombinant plasmids that contain deletions within the 145 bp AAV terminal repeats. When the plasmids were transfected into human cells, mutants that contained deletions within the left (type I) or right (type II) terminal repeat were viable. Of four mutants examined that contained deletions in both termini (type III), only one was viable. All of the viable mutants produced AAV virions that contained wild-type AAV DNA. Furthermore, the viable type III deletion could be converted to a nonviable mutant by deleting all copies of an 11 bp sequence from its termini. We conclude that there is an efficient mechanism for correcting deletions within the AAV termini. A model that could account for these observations is also discussed.
Tags: Human; Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.
Descriptors: *Dependovirus--genetics--GE; *Virus Replication; Chromosome Deletion; DNA Replication; DNA Transposable Elements; DNA, Recombinant; DNA, Viral--genetics--GE; Defective Viruses--genetics--GE; Mutation; Plasmids; Repetitive Sequences, Nucleic Acid
Molecular Sequence Databank No.: GENBANK/J01901; GENBANK/M12405; GENBANK/M12468; GENBANK/M12469
CAS Registry No.: 0 (DNA Transposable Elements); 0 (DNA, Recombinant); 0 (DNA, Viral); 0 (Plasmids)
Record Date Created: 19840928
? t s7/7/7
7/7/7
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.
06701124 91012793 PMID: 2145445

Interaction of virally coded protein and a cell cycle-regulated cellular protein with the bovine parvovirus left terminus ori.

Metcalf J B; Bates R C; Lederman M

Biology Department, Virginia Polytechnic Institute and State University, Blacksburg 24061-0406.

Journal of virology (UNITED STATES) Nov 1990, 64 (11) p5485-90, ISSN 0022-538X Journal Code: 0113724

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Replication of parvoviruses requires cis signals located in terminal palindromes that function as origins of replication in conjunction with trans-acting viral and cellular proteins. A gel retardation assay was used to identify proteins in crude nuclear extracts of bovine parvovirus (BPV)-infected bovine fetal lung cells that interact with the hairpinned left end (3' OH terminus of the viral minus strand in the flop conformation) of BPV. Three specific DNA-protein complexes formed. One complex was shown to involve a BPV structural protein(s) by inhibiting its formation when antiserum specific for these BPV proteins was used. By specific competition with serum containing antibodies against the BPV nonstructural proteins, a second complex was shown to involve a BPV nonstructural protein. A third complex contained protein of cellular origin and was also formed with extracts of uninfected bovine fetal lung cells. DNA competition assays suggest that the viral proteins do not bind to the right hairpin, which differs in sequence and secondary structure from the left terminus, or to a BPV terminus that lacks the first 52 nucleotides, preventing formation of the stem of the hairpin. The cellular protein is regulated in a cell cycle-dependent fashion, with its binding activity increased in uninfected, actively dividing cells compared with contact-inhibited cells. Since autonomous parvovirus replication requires an S-phase factor for progeny formation, the terminal binding protein demonstrated here is a candidate for this factor.

Record Date Created: 19901115

? t s7/7/2 3 12 13 16 17

7/7/2

DIALOG(R)File 155:MEDLINE(R)

10378075 99355161 PMID: 10428207

cis requirements for the efficient production of recombinant DNA vectors based on autonomous parvoviruses.

Kestler J; Neeb B; Struyf S; Van Damme J; Cotmore S F; D'Abramo A; Tattersall P; Rommelaere J; Dinsart C; Cornelis J J

Applied Tumor Virology Abt. F0100 and INSERM U375 Deutsches Krebsforschungszentrum, Heidelberg, Germany.

Human gene therapy (UNITED STATES) Jul 1 1999, 10 (10) p1619-32, ISSN 1043-0342 Journal Code: 9008950

Contract/Grant No.: CA29303; CA; NCI

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The replication of viral genomes and the production of recombinant viral vectors from infectious molecular clones of parvoviruses MVMp and H1 were greatly improved by the introduction of a consensus NS-1 nick site at the junction between the left-hand viral terminus and the plasmid DNA. Progressive deletions of up to 1600 bp in the region encoding the structural genes as well as insertions of foreign DNA in replacement of those sequences did not appreciably affect the replication ability of the recombinant H1 virus genomes. In contrast, the incorporation of these genomes into recombinant particles appeared to depend on in cis-provided structural gene sequences. Indeed, the production of H1 viral vectors by cotransfection of recombinant clones and helper plasmids providing the structural proteins (VPs) in trans, drastically decreased when more than 800 bp was removed from the VP transcription unit. Furthermore, titers of viral vectors, in which most of the VP-coding region was replaced by an equivalent-length sequence consisting of reporter cDNA and stuffer DNA, were reduced more than 50 times in comparison with recombinant vectors in which stuffer DNA was not substituted for the residual VP sequence. In addition, viral vector production was restricted by the overall size of the genome, with a mere 6% increase in DNA length leading to an approximately 10 times lower encapsidation yield. Under conditions fulfilling the above-mentioned requirements for efficient packaging, titers of virus vectors from improved recombinant molecular DNA clones amounted to 5 x 10(7) infectious units per milliliter of crude extract. These titers should allow the assessment of the therapeutic effect of recombinant parvoviruses expressing small transgenes in laboratory animals.

Record Date Created: 19990922

7/7/3

DIALOG(R)File 155:MEDLINE(R)

09588055 98007643 PMID: 9349487

The minute virus of mice (MVM) nonstructural protein NS1 induces nicking of MVM DNA at a unique site of the right-end telomere in both hairpin and duplex conformations in vitro.

Willwand K; Baldauf A Q; Deleu L; Mumtsidu E; Costello E; Beard P; Rommelaere J

Deutsches Krebsforschungszentrum, Department of Applied Tumor Virology, and Formation INSERM U375, Heidelberg, Germany. k.willwand@dkfz-heidelberg.de

Journal of general virology (ENGLAND) Oct 1997, 78 (Pt 10) p2647-55, ISSN 0022-1317 Journal Code: 0077340

Document type: Journal Article

Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

The right-end telomere of replicative form (RF) DNA of the autonomous parvovirus minute virus of mice (MVM) consists of a sequence that is self-complementary except for a three nucleotide loop around the axis of symmetry and an interior bulge of three unpaired nucleotides on one strand (designated the right-end 'bubble'). This right-end inverted repeat can exist in the form of a folded-back strand (hairpin conformation) or in an extended form, base-paired to a copy strand (duplex conformation). We recently reported that the right-end telomere is processed in an A9 cell extract supplemented with the MVM nonstructural protein NS1. This processing is shown here to result from the NS1-dependent nicking of the complementary strand at a unique position 21 nt inboard of the folded-back genomic 5' end. DNA species terminating in duplex or hairpin configurations, or in a mutated structure that has lost the right-end bulge, are all cleaved in the presence of NS1, indicating that features distinguishing these structures are not prerequisites for nicking under the in vitro conditions tested. Cleavage of the hairpin structure is followed by strand-displacement synthesis, generating the right-end duplex conformation, while processing of the duplex structure leads to the release of free right-end telomeres. In the majority of molecules, displacement synthesis at the right terminus stops a few nucleotides before reaching the end of the template strand, possibly due to NS1 which is covalently bound to this end. A fraction of the right-end duplex product undergoes melting and re-folding into hairpin structures (formation of a 'rabbit-ear' structure).

Record Date Created: 19971117

7/7/12
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.
06115323 89199654 PMID: 2539485

Interactions between the termini of adeno-associated virus DNA.

Bohenzky R A; Berns K I

Department of Immunology and Medical Microbiology, College of Medicine, University of Florida, Gainesville 32610.

Journal of molecular biology (ENGLAND) Mar 5 1989, 206 (1) p91-100, ISSN 0022-2836 Journal Code: 2985088R

Contract/Grant No.: AI-01770; AI; NIAID; AI-22251; AI; NIAID

Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

The adeno-associated virus (AAV) genome is a linear, single polynucleotide chain with inverted terminal repeats of 145 bases. In order to test whether the terminal repeats at opposite ends of the genome have to be able to completely base-pair during DNA replication, we have created chimeric genomes in which an 11 base symmetrical sequence has been deleted from the terminal repeat at one end of the genome and replaced by a different 12 base symmetrical sequence. We have used these chimeric constructs either as a duplex insert in pBR322 or as purified duplex virion DNA to transfect adenovirus-infected HeLa cells. When chimeric duplex virion DNA was used, all of the progeny virions obtained after two cell passages contained DNA with wild-type sequences in both terminal repeats. When plasmid clones were used, the structure of virion DNA depended on the original orientation. If the mutant terminal repeat was originally at the right end of the genome (terminus of genetic map), all progeny terminal repeat sequences were again wild-type. However, if the original construct contained the mutant sequence in the left terminal repeat, the majority of progeny molecules were parental in type (i.e. mutant left and wild-type right terminal repeat). We conclude (1) although the terminal repeats at opposite ends of the genome may interact during DNA replication, it is not necessary that they be perfectly complementary. (2) In direct competition, the wild-type sequence displays an advantage over the mutant allele. (3) In a plasmid clone, the terminal repeat on the left end of the genome is at an advantage in a competitive situation. We note that the left terminal repeat is adjacent to a transcriptional promoter.

Record Date Created: 19890525

7/7/13
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.
05899897 88333157 PMID: 2843676

Analysis of the termini of the DNA of bovine parvovirus: demonstration of sequence inversion at the left terminus and its implication for the replication model.

Chen K C; Shull B C; Lederman M; Stout E R; Bates R C

Department of Biology, Virginia Polytechnic Institute and State University, Blacksburg 24061.

Journal of virology (UNITED STATES) Oct 1988, 62 (10) p3807-13, ISSN 0022-538X Journal Code: 0113724

Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

The distribution of terminal-sequence orientations in the viral DNA of bovine parvovirus (BPV), an autonomous parvovirus, was studied by end labeling and restriction enzyme digestion and also by cloning. The left (3') end of the minus strand of BPV was found in two alternative sequence orientations (designated as flip and flop, which are reverse complements of each other), with a 10-fold excess of flip. This is in contrast to the autonomous rodent parvoviruses which encapsidate minus-strand DNA with only the flip orientation at this end. The right (5') end of the minus strand of

BPV contained both sequence orientations with equal frequencies, as in the rodent parvoviruses. Sequence inversions were also detected at both ends of the plus strand, which makes up about 10% of the encapsidated BPV DNA. Each terminus of BPV DNA had a characteristic ratio of flip to flop forms, and this ratio was restored in the progeny DNA resulting from transfection with genomic clones of different defined terminal conformations. Replicative-form DNA showed the same distribution of terminal-sequence orientations as the reannealed plus and minus virion DNAs, suggesting that the distribution of flip and flop forms observed in virion DNA is not due to selective encapsidation, but rather to the specific distribution of replicative forms. The current replication model for autonomous parvoviruses, which was based on the available data for the rodent parvoviruses, cannot account for the observed distribution of BPV DNA. An alternative model is suggested.
Record Date Created: 19881019

7/7/16
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.
04751162 85135064 PMID: 3973977
Sequence analysis of the termini of virion and replicative forms of minute virus of mice DNA suggests a modified rolling hairpin model for autonomous parvovirus DNA replication.
Astell C R; Chow M B; Ward D C
Journal of virology (UNITED STATES) Apr 1985, 54 (1) p171-7, ISSN 0022-538X Journal Code: 0113724
Contract/Grant No.: CA-16038; CA; NCI; GM-20124; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The nucleotide sequences of the terminal regions of monomer replicative form DNA, a pivotal intermediate species in the replication of minute virus of mice, were determined. The left (3') terminus had a unique sequence on both strands and in both 3'-hairpin configurations. In contrast, the right (5') terminus was sequence heterogeneous and extended an additional 18 base pairs beyond that expected from the known sequence of the virion DNA. These data unambiguously establish the sequence complexity at the termini of both the single-stranded viral genome and the pool of replicative DNA. A comparison of the combined sequence information leads us to propose a modified rolling hairpin model for the replication of autonomous parvoviruses which is compatible with all available data.
Record Date Created: 19850418

7/7/17
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.
04584837 84282662 PMID: 6088052
Rescue of adeno-associated virus from recombinant plasmids: gene correction within the terminal repeats of AAV.
Samulski R J; Srivastava A; Berns K I; Muzyczka N
Cell (UNITED STATES) May 1983, 33 (1) p135-43, ISSN 0092-8674 Journal Code: 0413066
Contract/Grant No.: 5 R01 AI16326; AI; NIAID; 5 T32 AI07110; AI; NIAID
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We have isolated three types of pBR322-AAV recombinant plasmids that contain deletions within the 145 bp AAV terminal repeats. When the plasmids were transfected into human cells, mutants that contained deletions within the left (type I) or right (type II) terminal repeat were viable. Of four mutants examined that contained deletions in both termini (type III), only one was viable. All of the viable mutants produced AAV virions that contained wild-type AAV DNA. Furthermore, the viable type III deletion could be converted to a nonviable mutant by deleting all copies of an 11 bp sequence from its termini. We conclude that there is an efficient mechanism for correcting deletions within the AAV termini. A model that could account for these observations is also discussed.
Record Date Created: 19840928
? t s9/7/6 7
9/7/6
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.
09268918 97151079 PMID: 8995615
Specific initiation of replication at the right-end telomere of the closed species of minute virus of mice replicative-form DNA.
Baldauf A Q; Willwand K; Mumtsidu E; Nuesch J P; Rommelaere J
Department of Applied Tumor Virology, Deutches Krebsforschungszentrum, Heidelberg, Germany.
Journal of virology (UNITED STATES) Feb 1997, 71 (2) p971-80, ISSN 0022-538X Journal Code: 0113724
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We have developed an in vitro system that supports the replication of natural DNA templates of the autonomous parvovirus minute virus of mice (MVM). MVM virion DNA, a single-stranded molecule bracketed by short, terminal, self-complementary sequences, is converted into double-stranded replicative-form (RF) DNA when incubated in mouse A9 fibroblast extract. The 3' end of the newly synthesized complementary strand is ligated to the right-end hairpin of the virion strand, resulting in the formation of a covalently closed RF (cRF) molecule as the major conversion product. cRF

DNA is not further replicated in A9 cell extract alone. On addition of purified MVM nonstructural protein NS1 expressed from recombinant baculoviruses or vaccinia viruses, cRF DNA is processed into a right-end (5' end of the virion strand) extended form (5'eRF). This is indicative of NS1-dependent nicking of the right-end hairpin at a distinct position, followed by unfolding of the hairpin and copying of the terminal sequence. In contrast, no resolution of the left-end hairpin can be detected in the presence of NS1. In the course of the right-end nicking reaction, NS1 gets covalently attached to the right-end telomere of the DNA product, as shown by immunoprecipitation with NS1-specific antibodies. The 5'eRF product is the target for additional rounds of NS1-induced nicking and displacement synthesis at the right end, arguing against the requirement of the hairpin structure for recognition of the DNA substrate by NS1. Further processing of the 5'eRF template in vitro leads to the formation of dimeric RF (dRF) DNA in a left-to-left-end configuration, presumably as a result of copying of the whole molecule by displacement synthesis initiated at the right-end telomere. Formation of dRF DNA is highly stimulated by NS1. The experimental results presented in this report support various assumptions of current models of parvovirus DNA replication and provide new insights into the replication functions of the NS1 protein.

Record Date Created: 19970218

9/7/7
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.
08223506 94357188 PMID: 8076610

An asymmetric nucleotide in the parvoviral 3' hairpin directs segregation of a single active origin of DNA replication.

Cotmore S F; Tattersall P

Department of Laboratory Medicine, Yale University School of Medicine, New Haven, CT 06510.

EMBO journal (ENGLAND) Sep 1 1994, 13 (17) p4145-52, ISSN 0261-4189
Journal Code: 8208664

Contract/Grant No.: AI26109; AI; NIAID; CA29303; CA; NCI

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The 3' telomere of the linear single-stranded DNA genome of minute virus of mice (MVM), a murine parvovirus, can assume a complex hairpin structure. This contains a stem in which there is a mismatched 'bubble' sequence where a GA doublet opposes a GAA triplet. During replication, this hairpin is copied to form an imperfect palindrome which bridges adjacent genomes in a dimer duplex intermediate, leaving the two 'bubble' sequences embedded in potential replication origins on either side of the axis of symmetry. Such junctions are resolved asymmetrically in vitro in a DNA synthetic reaction which requires the viral initiator protein NS1. We show that the sequence surrounding the doublet is a potent origin, but the analogous region containing the triplet is completely inactive. The active origin is approximately 50 bp long, extending from an Activated Transcription Factor binding site at one end to a position some 7 bp beyond the major initiation site, to which NS1 ultimately becomes covalently attached. The actual sequence of the GA doublet is unimportant, but insertion of any third nucleotide here inactivates the origin, indicating that it represents a critical spacer element. Segregation of this asymmetry, therefore, allows the virus to confine replication initiation to one particular telomeric configuration.

Record Date Created: 19941005

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File 50:CAB Abstracts 1972-2003/Jan
     (c) 2003 CAB International
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See Help News50 for details.

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         176 S1
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00962529  CAB Accession Number: 802261627
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Tropical veterinary science. Research report 1979.
Publication Year: 1979
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File 50:CAB Abstracts 1972-2003/Jan
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*File 50: Truncating CC codes is recommended for full retrieval.
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2/7/85 (Item 11 from file: 50)
DIALOG(R)File 50:CAB Abstracts
(c) 2003 CAB International. All rts. reserv.
03013408 CAB Accession Number: 952206035
Structure, sequence, and function correlations among parvoviruses.
Chapman, M. S.; Rossmann, M. G.
Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907, USA.
Virology (New York) vol. 194 (2): p.491-508
Publication Year: 1993
ISSN: 0042-6822 --
Language: English
Document Type: Journal article
83 ref.

2/7/91 (Item 17 from file: 50)
DIALOG(R)File 50:CAB Abstracts
(c) 2003 CAB International. All rts. reserv.
02467824 CAB Accession Number: 910504273
Features of organization of the genome of the densoviruses.
Galev, E. E.; Afanas'ev, B. N.; Buchatskii, L. P.; Kozlov, Yu. V.; Baev, A. A.
V.A. Engel'gardt Institute of Molecular Biology, Academy of Sciences of the USSR, Moscow, RSFSR, USSR.
Doklady, Biological Sciences vol. 307 (1-6): p.537-541
Publication Year: 1989, publ. 1990
ISSN: 0012-4966
Translated from Doklady Akademii Nauk SSSR, 307: 996-1000 (1989) --
Language: English
Document Type: Journal article
The nucleotide sequence is given of the virion of the densonucleosis virus (densovirus) of mosquitoes (MDV), a member of the Parvoviridae which includes the smallest known viruses pathogenic to insects. This virus infects the larvae of Culex, Aedes and Culiseta spp. and the insecticide preparation Viroden has been developed from it. The present paper shows the sequence of the DNA "plus" chain and 3 extended open reading frames (ORFs), with the probable promoters and polyadenylation signals for these ORFs underlined, the 3'-terminal sequence of the virion "minus" chain of MDV DNA with the AUG codon of the 1st ORF underlined, and the region of greatest homology of the NS1 proteins of MDV and other parvoviruses including human parvovirus (HPV), adeno-associated virus 2 (AAV2), bovine parvovirus (BVP), Aleutian disease (parvo)virus of mink (ADV) and minute (parvo)virus of mice (MVM). Studies are underway to determine the complete primary structure of MDV. 15 ref.
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S1 250 BOVINE(W)PARVO?
S2 194 RD (unique items)
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188736 ORIGIN
108039 REPLICATION
S3 4946 ORIGIN(2N)REPLICATION
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43 ADENOASSOCIAT?
2729 ADENO
1384056 ASSOCIAT?
1647 ADENO(W)ASSOCIAT?

S4 12290 PARVO? OR AAV OR ADENOASSOCIAT? OR ADENO(W)ASSOCIAT?
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12290 S4
S5 59 S3 AND S4
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6/7/8 (Item 8 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.
12699923 21574575 PMID: 11707592
Adeno-associated virus (AAV) site-specific recombination does not require a Rep-dependent origin of replication within the AAV terminal repeat.
Young S M; Samulski R J
Curriculum in Genetics and Molecular Biology, Gene Therapy Center, University of North Carolina, Chapel Hill, NC 27599, USA.
Proceedings of the National Academy of Sciences of the United States of America (United States) Nov 20 2001, 98 (24) p13525-30, ISSN 0027-8424 Journal Code: 7505876
Contract/Grant No.: DK51880; DK; NIDDK; HL 48347; HL; NHLBI
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Adeno-associated virus (AAV) is the only known eukaryotic virus capable of targeted integration in human cells. An AAV Rep binding element (RBE) and terminal resolution site (trs) identical to the viral terminal repeats required for AAV DNA replication are located on chromosome (ch) 19. Both ch-19 RBE and trs elements have been shown to be essential for viral targeting to this locus. To characterize the role of the AAV inverted terminal repeat (ITR) cis-acting sequences in targeted integration an AAV trs mutant incapable of supporting viral replication was tested. Wild-type and mutant substrates were assayed for targeted integration after cotransfection in the presence or absence of Rep. Our results demonstrated that, in the presence of Rep78, both ITR substrates targeted to ch-19 with similar frequency. Molecular characterization of the mutant ITR integrants confirmed the presence of the trs mutation in the majority of samples tested. Complementation analysis confirmed that the mutant targeted viral genomes were unable to rescue and replicate. In addition, Rep78 induced extensive rearrangement and amplification of ch-19 sequences independent of wild-type or mutant targeting substrate. These studies demonstrate that Rep-dependent nicking of the viral cis-acting trs sequence is not a prerequisite for site-specific recombination and suggests AAV targeting is mediated by Rep78/68-dependent replication from the ch-19 origin of replication (ori). These studies have significant impact toward the understanding of AAV site-specific recombination and the development of targeting vectors.
Record Date Created: 20011121

6/7/9 (Item 9 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.
11292356 21329483 PMID: 11435581
Minute virus of mice initiator protein NS1 and a host KDWK family transcription factor must form a precise ternary complex with origin DNA for nicking to occur.
Christensen J; Cotmore S F; Tattersall P
Institute of Medical Microbiology and Immunology, University of Copenhagen, Panum Institute, Copenhagen 2200 N, Denmark.
Journal of virology (United States) Aug 2001, 75 (15) p7009-17, ISSN 0022-538X Journal Code: 0113724
Contract/Grant No.: AI26109; AI; NIAID
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Parvoviral rolling hairpin replication generates palindromic genomic concatemers whose junctions are resolved to give unit-length genomes by a process involving DNA replication initiated at origins derived from each viral telomere. The left-end origin of minute virus of mice (MVM), oriL, contains binding sites for the viral initiator nickase, NS1, and parvovirus initiation factor (PIF), a member of the emerging KDWK family of transcription factors. oriL is generated as an active form, oriL(TC), and as an inactive form, oriL(GAA), which contains a single additional nucleotide inserted between the NS1 and PIF sites. Here we examined the interactions on oriL(TC) which lead to activation of NS1 by PIF. The two subunits of PIF, p79 and p96, cooperatively bind two ACGT half-sites, which can be flexibly spaced. When coexpressed from recombinant baculoviruses, the PIF subunits preferentially form heterodimers which, in the presence of ATP, show cooperative binding with NS1 on oriL, but this interaction is preferentially enhanced on oriL(TC) compared to oriL(GAA). Without ATP, NS1 is unable to bind stably to its cognate site, but PIF facilitates this interaction, rendering the NS1 binding site, but not the nick site, resistant to DNase I. Varying the spacing of the PIF half-sites shows that the distance between the NS1 binding site and the NS1-proximal half-site is critical for nickase activation, whereas the position of the distal half-site is unimportant. When expressed separately, both PIF subunits form homodimers that bind site specifically to oriL, but only complexes containing p79 activate the NS1 nickase function.
Record Date Created: 20010703

6/7/20 (Item 20 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09925898 98362103 PMID: 9696794
Packaging cells based on inducible gene amplification for the production of adeno-associated virus vectors.
Inoue N; Russell D W
Markey Molecular Medicine Center and Department of Medicine, University of Washington, Seattle, Washington 98195, USA.
Journal of virology (UNITED STATES) Sep 1998, 72 (9) p7024-31, ISSN 0022-538X Journal Code: 0113724
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Although vectors based on adeno-associated virus (AAV) offer several unique advantages, their usage has been hampered by the difficulties encountered in vector production. In this report, we describe a new AAV packaging system based on inducible amplification of integrated helper and vector constructs containing the simian virus 40 (SV40) replication origin. The packaging and producer cell lines developed express SV40 T antigen under the control of the reverse tetracycline transactivator system, which allows inducible amplification of chromosomal loci linked to the SV40 origin. Culturing these cells in the presence of doxycycline followed by adenovirus infection resulted in helper and vector gene amplification as well as higher vector titers. Clonal producer cell lines generated vector titers that were 10 times higher than those obtained by standard methods, with approximately 10(4) vector particles produced per cell. These stocks were free of detectable replication-competent virus. The lack of a transfection step combined with the reproducibility of stable producer lines makes this packaging method ideally suited for the large-scale production of vector stocks for human gene therapy.
Record Date Created: 19980916

6/7/32 (Item 32 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08538652 95297175 PMID: 7778304
Minimum origin requirements for linear duplex AAV DNA replication in vitro.
Ward P; Berns K I
Department of Microbiology, W. R. Hearst Microbiology Research Center, Cornell University Medical College, New York, New York 10021, USA.
Virology (UNITED STATES) Jun 1 1995, 209 (2) p692-5, ISSN 0042-6822 Journal Code: 0110674
Contract/Grant No.: GM50023; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We have investigated the minimal requirements for a functional origin of DNA replication in an in vitro assay which requires the adeno-associated virus (AAV) Rep 68/78 protein. When a linear duplex template was used, initiation of one round of DNA replication was achieved when nucleotides 1-56 of the AAV inverted terminal repeat (in the flop orientation) were present at the terminus of the template. A terminal resolution site was not required. Deletion of an additional 11 nucleotides from the terminal repeat blocked the reaction.
Record Date Created: 19950711

6/7/35 (Item 35 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08223506 94357188 PMID: 8076610
An asymmetric nucleotide in the parvoviral 3' hairpin directs segregation of a single active origin of DNA replication.
Cotmore S F; Tattersall P
Department of Laboratory Medicine, Yale University School of Medicine, New Haven, CT 06510.
EMBO journal (ENGLAND) Sep 1 1994, 13 (17) p4145-52, ISSN 0261-4189 Journal Code: 8208664
Contract/Grant No.: AI26109; AI; NIAID; CA29303; CA; NCI
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The 3' telomere of the linear single-stranded DNA genome of minute virus of mice (MVM), a murine parvovirus, can assume a complex hairpin structure. This contains a stem in which there is a mismatched 'bubble' sequence where a GA doublet opposes a GAA triplet. During replication, this hairpin is copied to form an imperfect palindrome which bridges adjacent genomes in a dimer duplex intermediate, leaving the two 'bubble' sequences embedded in potential replication origins on either side of the axis of symmetry. Such junctions are resolved asymmetrically in vitro in a DNA synthetic reaction which requires the viral initiator protein NS1. We show that the sequence surrounding the doublet is a potent origin, but the analogous region containing the triplet is completely inactive. The active origin is approximately 50 bp long, extending from an Activated Transcription Factor binding site at one end to a position some 7 bp beyond the major initiation site, to which NS1 ultimately becomes covalently attached. The actual sequence of the GA doublet is unimportant, but insertion of any third nucleotide here inactivates the origin, indicating that it represents a critical spacer element. Segregation of this asymmetry, therefore, allows the virus to confine replication initiation to one particular telomeric

configuration.
Record Date Created: 19941005

6/7/38 (Item 38 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.
07850201 93381812 PMID: 8396670
Features of the adeno-associated virus origin involved in substrate recognition by the viral Rep protein.
Snyder R O; Im D S; Ni T; Xiao X; Samulski R J; Muzyczka N
Department of Microbiology, State University of New York at Stony Brook Medical School 11794-8621.
Journal of virology (UNITED STATES) Oct 1993, 67 (10) p6096-104, ISSN 0022-538X Journal Code: 0113724
Contract/Grant No.: 5 PO1 CA2814607; CA; NCI; AI25530; AI; NIAID; RO1 GM3572302; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We previously demonstrated that the adeno-associated virus (AAV) Rep68 and Rep78 proteins are able to nick the AAV origin of DNA replication at the terminal resolution site (trs) in an ATP-dependent manner. Using four types of modified or mutant substrates, we now have investigated the substrate requirements of Rep68 in the trs endonuclease reaction. In the first kind of substrate, portions of the hairpinned AAV terminal repeat were deleted. Only deletions that retained virtually all of the small internal palindromes of the AAV terminal repeat were active in the endonuclease reaction. This result confirmed previous genetic and biochemical evidence that the secondary structure of the terminal repeat was an important feature for substrate recognition. In the second type of substrate, the trs was moved eight bases further away from the end of the genome. The mutant was nicked at a 50-fold-lower frequency relative to a wild-type origin, and the nick occurred at the correct trs sequence despite its new position. This finding indicated that the endonuclease reaction required a specific sequence at the trs in addition to the correct secondary structure. It also suggested that the minimum trs recognition sequence extended three bases from the cut site in the 3' direction. The third type of substrate harbored mismatched base pairs at the trs. The mismatch substrates contained a wild-type sequence on the strand normally cut but an incorrect sequence on the complementary strand. All of the mismatch mutants were capable of being nicked in the presence of ATP. However, there was substantial variation in the level of activity, suggesting that the sequence on the opposite strand may also be recognized during nicking. Analysis of the mismatch mutants also suggested that a single-stranded trs was a viable substrate for the enzyme. This interpretation was confirmed by analysis of the fourth type of substrate tested, which contained a single-stranded trs. This substrate was also cleaved efficiently by the enzyme provided that the correct strand was present in the substrate. In addition, the single-stranded substrate no longer required ATP as a cofactor for nicking. Finally, all of the substrates with mutant trss bound the Rep protein as efficiently as the wild-type did. This finding indicated that the sequence at the cut site was not involved in recognition of the terminal repeat for specific binding by the enzyme. We concluded that substrate recognition by the AAV Rep protein involves at least two and possibly as many as four features of the AAV terminal repeat.(ABSTRACT TRUNCATED AT 400 WORDS)
Record Date Created: 19931012

6/7/48 (Item 48 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.
04676844 85058177 PMID: 6094825
Replication of adeno-associated virus DNA. Complementation of naturally occurring rep- mutants by a wild-type genome or an ori- mutant and correction of terminal palindrome deletions.
Senapathy P; Tratschin J D; Carter B J
Journal of molecular biology (ENGLAND) Oct 15 1984, 179 (1) p1-20, ISSN 0022-2836 Journal Code: 2985088R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
When the entire adeno-associated virus (AAV) genome is inserted into a bacterial plasmid, infectious AAV genomes can be rescued and replicated when the recombinant AAV-plasmid DNA is transfected into human 293 cells together with helper adenovirus particles. We have taken advantage of this experimental system to analyze the effects of several classes of mutations on replication of AAV DNA. We obtained AAV mutants by molecular cloning in bacterial plasmids of naturally occurring AAV variant or defective-interfering genomes. Each of these mutants contains a single internal deletion of AAV coding sequences. Also, some of these mutant-AAV plasmids have additional deletions of one or both AAV terminal palindromes introduced during constructions in vitro. We show here that AAV mutants containing internal deletions were defective for replicative form DNA replication (rep-) but could be complemented by intact wild-type AAV. This indicates that an AAV replication function, Rep, is required for normal AAV replication. Mutants in which both terminal palindromes were deleted (ori-) were also replication defective but were not complementable by wild-type AAV. The cis-dominance of the ori- mutation shows that the replication origin is comprised in part of the terminal palindrome. Deletion of only one terminal palindrome was phenotypically wild-type and allowed rescue and replication of AAV genomes in which the deleted region was regenerated apparently by an intramolecular correction mechanism. One model for this

correction mechanism is proposed. An AAV ori- mutant also complemented replication of AAV rep- mutants as efficiently as did wild-type AAV. These studies also revealed an unexpected additional property of the deletion mutants in that monomeric single-stranded single-stranded DNA accumulated very inefficiently even though monomeric single-stranded DNA from the complementing wild-type AAV did accumulate.
Record Date Created: 19841228

6/7/50 (Item 50 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.
03968667 82242308 PMID: 6284985
DNA sequence of the 5' terminus containing the replication origin of parvovirus replicative form DNA.
Rhode S L; Klaassen B
Journal of virology (UNITED STATES) Mar 1982, 41 (3) p990-9, ISSN 0022-538X Journal Code: 0113724
Contract/Grant No.: CA-25866; CA; NCI; CA26801; CA; NCI
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The nucleotide sequence of the 5' terminus of the parvovirus H-1 was determined. There are two orientations of the 242-base-pair terminal palindrome in native replicative form DNA, one inverted with respect to the other. Adjacent to the terminal palindrome is an AT-rich region that is noncoding and contains a 55-base-pair tandem repeat. The addition mutant of H-1, DI-1, was also sequenced in this region and shown to have three copies of the tandem repeat sequence. Similarly, the related parvovirus H-3 contains only one copy of this repeat sequence. This region contains the replication origin for parvovirus replicative form DNA replication. Some of the implications of these results are discussed.
Record Date Created: 19820910

6/7/51 (Item 51 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.
03332242 80162970 PMID: 232178
Incomplete genomes of the parvovirus minute virus of mice: selective conservation of genome termini, including the origin for DNA replication.
Faust E A; Ward D C
Journal of virology (UNITED STATES) Oct 1979, 32 (1) p276-92, ISSN 0022-538X Journal Code: 0113724
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Deletion mutants of minute virus of mice arising during a single high-multiplicity passage and after serial undiluted passage have been isolated, and the incomplete viral genomes contained therein have been analyzed. The DNA isolated from incomplete virions derived from a single high-multiplicity passage was heterogeneous, ranging in size from 15 to 70% of the intact viral genome, with an average molecular length of approximately, 2,000 nucleotides. Two distinct types of molecules, designated as type I D-DNA and type II D-DNA, could be distinguished on the basis of their degree of secondary structure, and these were present in roughly equal amounts. Type I D-DNAs were predominantly single-stranded, recombinant molecules in which the self-complementary sequences derived from both genomic termini were conserved. The 5' terminus was modified relative to the analogous wild-type structure. Although virtually all of the wild-type genome sequence was seen in the total type I D-DNA population, sequences which map between coordinates 47.3 and 87.1 were clearly underrepresented. However, the extent and position of the deletions in individual molecules varied significantly. The shortest molecules in the population lacked between 90 and 95% of the internal wild-type genome sequence and consisted of sequences derived almost exclusively from within 5.0 map units (250 nucleotides) at both ends of the viral genome. Moreover, these miniature recombinant molecules were selectively amplified during serial undiluted passage and were therefore believed to contain all of the critical recognition sites necessary for the replication of minute virus of mice viral DNA. Type II D-DNAs were virus-specific, double-stranded hairpin molecules whose complementary strands were covalently continuous at variable sites distal to the 5' end of the viral minus strand. In sharp contrast to the type I genomes, these hairpin molecules consisted of sequences which mapped entirely at the 5' end of the viral genome between positions 85.0 and 100. Furthermore, type II molecules were gradually lost from the total D-DNA population during serial undiluted passage, suggesting that these molecules are not competent for DNA Replication but arise as the result of fatal replication errors. Deletion mutants of the type described here for minute virus of mice should be valuable generally as aids to future studies on parvovirus DNA replication, transcription, and cell-virus interactions.
Record Date Created: 19800616

6/7/52 (Item 52 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.
03192159 80009430 PMID: 225552
Nucleotide sequence of the self-priming 3' terminus of the single-stranded DNA extracted from the parvovirus Kilham rat virus.
Salzman L A; Fabisch P
Journal of virology (UNITED STATES) Jun 1979, 30 (3) p946-50, ISSN 0022-538X Journal Code: 0113724
Document type: Journal Article

Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The parvovirus genome is a linear, single-stranded DNA molecule with double-stranded hairpin termini. The 3' terminus can serve in vitro as a self-primer for the synthesis of a double-stranded viral DNA intermediate. We have sequenced the nucleotides in the 3' terminus and propose a model for the secondary structure of the terminus and the in vitro origin of replication for the complementary viral DNA strand.
Record Date Created: 19791128
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? ds
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S1 250 BOVINE(W)PARVO?
S2 194 RD (unique items)
S3 4946 ORIGIN(2N)REPLICATION
S4 12290 PARVO? OR AAV OR ADENOASSOCIAT? OR ADENO(W)ASSOCIAT?
S5 59 S3 AND S4
S6 55 RD (unique items)
? b 357
05mar03 11:43:58 User208669 Session D2223.4
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$0.00 55 Type(s) in Format 6
$2.10 10 Type(s) in Format 7
$2.10 65 Types
$5.18 Estimated cost File155
$3.62 0.804 DialUnits File50
$0.00 120 Type(s) in Format 6
$4.00 2 Type(s) in Format 7
$4.00 122 Types
$7.62 Estimated cost File50
OneSearch, 2 files, 1.767 DialUnits FileOS
$3.02 TELNET
$15.82 Estimated cost this search
$29.74 Estimated total session cost 4.248 DialUnits

File 357:Derwent Biotech Res. _1982-2003/Mar W2
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*File 357: File is now current. See HELP NEWS 357.
Alert feature enhanced for multiple files, etc. See HELP ALERT.

Set Items Description
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? parvo? or aav or adenoassociat? or adeno(w)associat?
Ref Items Index-term
E1 11 ARVILLA
E2 1 ARVIN
E3 0 *ARVO? OR AAV OR ADENOASSOCIAT? OR ADENO(W)ASSO
E4 1 ARVR
E5 1 ARV2
E6 2 ARW
E7 1 ARW-7
E8 11 ARX
E9 1 ARXIDA
E10 2 ARXIELLA
E11 30 ARXULA
E12 1 ARXXARZ
Enter P or PAGE for more
? s parvo? or aav or adenoassociat? or adeno(w)associat?
1291 PARVO?
610 AAV
12 ADENOASSOCIAT?
5299 ADENO
19569 ASSOCIAT?
1250 ADENO(W)ASSOCIAT?
S1 1542 PARVO? OR AAV OR ADENOASSOCIAT? OR ADENO(W)ASSOCIAT?
? s terminus or termini or telomer?
3786 TERMINUS
799 TERMINI
465 TELOMER?
S2 4872 TERMINUS OR TERMINI OR TELOMER?
? s s1 and s2
1542 S1
4872 S2
S3 45 S1 AND S2
? ds
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S1 1542 PARVO? OR AAV OR ADENOASSOCIAT? OR ADENO(W)ASSOCIAT?
S2 4872 TERMINUS OR TERMINI OR TELOMER?
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1595 LEFT
890 RIGHT
S4 2114 LEFT OR RIGHT
? s s1 and s4
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2114 S4
S5 58 S1 AND S4
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S6 55 S5 NOT S3
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S1 1542 PARVO? OR AAV OR ADENOASSOCIAT? OR ADENO(W)ASSOCIAT?
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S6 55 S5 NOT S3
S7 1772 ORIGIN (2N)REPLICATION
? s s1 and s7
1542 S1
1772 S7
S8 46 S1 AND S7
? t s8/7/1 4 18 19 27
8/7/1
DIALOG(R)File 357:Derwent Biotech Res.
(c) 2003 Thomson Derwent & ISI. All rts. reserv.
0302115 DBR Accession No.: 2003-03900 PATENT
Cis-acting replication elements from an Adeno-Associated Virus (AAV), useful for producing cell lines that express AAVs - recombinant adeno-associated virus production by packaging cell culture with potential application in gene therapy
AUTHOR: SALVETTI A; CHADEUF G; TESSIER J; MOULLIER P; LINDEN M R; WARD P; EPSTEIN A L
PATENT ASSIGNEE: UNIV NANTES 2002
PATENT NUMBER: WO 200246359 PATENT DATE: 20020613 WPI ACCESSION NO.: 2002-706808 (200276)
PRIORITY APPLIC. NO.: US 251576 APPLIC. DATE: 20001207
NATIONAL APPLIC. NO.: WO 2001EP15418 APPLIC. DATE: 20011206
LANGUAGE: English
ABSTRACT: DERWENT ABSTRACT: NOVELTY - An isolated nucleic acid sequence (I) comprising a first DNA sequence comprising a cis-acting replication element (CARE) from an Adeno-Associated Virus (AAV), and a second DNA sequence operably linked to the CARE, is new. Amplification of the isolated nucleic acid sequence occurs when the isolated nucleic acid sequence is integrated in the genome of a cell and the cell is contacted with a CARE-dependent replication inducer (CARE-DRI). DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) a method (II) for the amplification of a DNA sequence in a cell, comprising: (a) operably linking a DNA sequence to an isolated CARE; (b) introducing the sequence operably linked to the CARE into the cell genome; and (c) contacting the cell with a CARE-DRI; (2) a method (III) for the amplification of a DNA sequence operably linked to a CARE and integrated into the genome of a cell, comprising contacting the cell with a CARE-DRI; (3) a method (IV) for the amplification of a DNA sequence operably linked to a CARE and integrated into the genome of a cell, comprising contacting the cell with a CARE-DRI (the DNA sequence to be amplified encodes the cap genes of an Adeno-Associated Virus); (4) a highly producing rAAV packaging cell-line (V) comprising: (a) an integrated copy of the rep and cap genes, operably linked to a CARE; and (b) an integrated copy of an MV-derived vector, comprising a DNA sequence of interest flanked by AAV Inverted Terminal Repeats (ITRs) (replication of the integrated rep and cap genes is inducible by a CARE-DRI); (5) a highly producing rAAV packaging cell-line (VI) comprising: (a) an integrated copy of the rep and cap genes, operably linked to a CARE sequence; and (b) a second integrated copy of the cap gene; (6) a cell-line (VII) comprising an integrated CARE sequence operably linked to a DNA sequence heterologous to AAV and to the cells from which the cell-line is derived; (7) a method (VIII) of producing recombinant AAV preparations, comprising contacting cells harboring rep and cap genes operably linked to a CARE sequence with a CARE-DRI; (8) a method (IX) of producing recombinant AAV preparation, comprising transfecting the cell-line (VI) with a plasmid harboring a rAAV genome comprising a CARE, and contacting the cell-line with a CARE-DRI; and (9) a kit (X) for amplifying a DNA sequence in a cell, comprising the nucleic acid (I) and a CARE-DRI. BIOTECHNOLOGY - Preferred Nucleic Acid Sequences: In (I) the CARE comprises a defined nucleotide sequence (N1) given in the specification (or a fragment/mutant, provided the mutant/fragment still promotes the amplification of a DNA sequence integrated into the genome of a cell and operably linked to the CARE, following contacting the cell with a CARE-DRI. (I) Comprises a CARE and a polynucleotide sequence heterologous to AAV, a poly-linker comprising several cloning sites and/or genetic elements from a virus. (I) Comprises retroviral Long Terminal Repeats (LTRs). Preferred Methods: In (II) and (III) the cell is a cell-line harboring part of human papilloma virus selected from HeLa, Hela32, SIHA, CASKI cells and cells derived from them. In (III) the CARE-DRI is selected from Adenoviruses, Herpes viruses, the adenoviral DNA-Binding Protein (Ad DBP), the gene of the Ad DBP, and any gene transfer vector expressing the Ad DBP. In (IV) the DNA sequence to be amplified further encodes the rep genes of an Adeno Associated Virus. In the method (VII) the cells are the cell-lines (V) and/or (VI). In the methods (VIII) and (IX) the CARE-DRI is selected from Adenoviruses, Herpes viruses (preferred), the adenoviral DNA-Binding Protein (Ad DBP), the gene of the Ad DBP, and any gene transfer vector expressing the Ad DBP. In particular, the CARE-DRI is a herpesvirus mutant selected from DELTAICPO, HP66, HR94, and 1178ts. Preferred Cell-Lines: In (V) the AAV-derived vector comprises a CARE sequence, in sense or antisense orientation. The CARE linked to the integrated rep and cap genes is in sense orientation, and

the CARE comprised in the integrated rAAV vector is in antisense orientation. The packaging cell-line (V) may further comprises a second integrated copy of the cap gene operably linked to a CARE sequence. In the packaging cell-line (VI) the second integrated copy of the cap gene is operably linked to a CARE sequence. (V) And (VI) are derived from a human cell-line harboring part of human papilloma virus such as HeLa, HeLa32, SIHA and CASKI cells. In the cell-lines (V) - (VII) 1 or more of the integrated elements is flanked by retroviral Long Terminal Repeats (LTRs). Preferred Kits: In the kit (X) the CARE-DRI is selected from Adenoviruses, Herpes viruses, the adenoviral DNA-Binding Protein (Ad DBP), the gene of the Ad DBP, and any gene transfer vector expressing the Ad DBP. The kit further comprises a rep expression cassette enclosed in a plasmid or in a vector selected from the group comprising Adenoviruses, Herpes viruses and Retroviruses. The kit may further comprises a purified Rep protein. Preparation: The nucleic acid sequence (I) may be produced via standard recombinant and synthetic methodologies. USE - The nucleic acid (I) may be used in the production of packaging cell lines that express recombinant AAV. ADVANTAGE - The nucleic acid (I), derived from the genome of AAV-2 behaves like a replication origin in the presence of AAV Rep proteins and a helper virus (adenovirus). EXAMPLE - No example given.(76 pages)

8/7/4
DIALOG(R)File 357:Derwent Biotech Res.
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0297472 DBR Accession No.: 2002-19319
A cis-acting element that directs circular adeno-associated virus replication and packaging - adeno-associated virus replication and packaging, expression in cell culture use in gene therapy
AUTHOR: MUSATOV S; ROBERTS J; PFAFF D; KAPLITT M
CORPORATE AFFILIATE: Cornell Univ Rockefeller Univ
CORPORATE SOURCE: Kaplitt M, Cornell Univ, Weill Med Coll, Dept Neurosurg, 525 E 68 St, New York, NY 10021 USA
JOURNAL: JOURNAL OF VIROLOGY (76, 24, 12792-12802) 2002
ISSN: 0022-538X
LANGUAGE: English
ABSTRACT: AUTHOR ABSTRACT - A novel pathway of adeno-associated virus (AAV)
replication marked by the assembly of circular monomer duplex intermediates (cAAV) has been recently discovered. In the present report we identify a single AD domain of the inverted terminal repeat as a minimal origin of cAAV replication. A small internal palindrome (BB'), necessary for optimal Rep-inverted terminal repeat interaction, does not contribute to the efficiency of cAAV replication, whilethe terminal resolution site is an essential cis-acting element. Furthermore, recombinant cAAV vectors that encompass only the AD domain replicate exclusively in a circular form and no detectable linear duplex replicative intermediates are generated, suggesting that both pathways of AAV replicationare independent and can be separated. In addition, we show that cAAVs areefficient templates for encapsidation of single-stranded DNA genomes, an observation that assigns a biological role for these novel replication species. Together, these findings shed new light on the current model of AAV replication and packaging. (11 pages)

8/7/18
DIALOG(R)File 357:Derwent Biotech Res.
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0238719 DBR Accession No.: 99-08820 PATENT
Transcriptionally-activated adeno-associated virus inverted terminal repeat - recombinant adeno-associated virus vector-mediated chloramphenicol-acetyltransferase gene transfer and expression in human bronchial epithelium cell culture for gene therapy
AUTHOR: Feldhaus A L
CORPORATE SOURCE: Seattle, WA, USA.
PATENT ASSIGNEE: Targeted-Genet. 1999
PATENT NUMBER: WO 9920773 PATENT DATE: 990429 WPI ACCESSION NO.: 99-288312 (9924)
PRIORITY APPLIC. NO.: US 955400 APPLIC. DATE: 971021
NATIONAL APPLIC. NO.: WO 98US21937 APPLIC. DATE: 981020
LANGUAGE: English
ABSTRACT: A polynucleotide (PN) containing a transcriptionally-activated adeno-associated virus (AAV) inverted terminal repeat (ITR) (less than 400 bp in length) is new. Also claimed are: a PN consisting a transcriptionally-activated ITR and a second ITR chosen from a wild-type ITR, a transcriptionally-activated ITR, a D sequence, a trs or a portion of a wild-type ITR; a plasmid containing a PN and an element selected from the group of a replication origin and a reporter gene; a PN, further consisting a gene operably linked to a transcriptionally-activated ITR; an AAV virus particle containing any PN; a mammalian cell containing any PN; and a method of packaging a recombinant AAV vector. The transcriptionally-activated ITRs may be useful for the production of improved recombinant AAV vectors which may be particularly useful for the packaging of large transgenes, especially the cystic fibrosis transmembrane conductance regulator gene of cystic fibrosis gene therapy. In an example, recombinant AAV vectors containing the chloramphenicol-acetyltransferase (EC-2.3.1.28) gene, were used to transfect human bronchial epithelial IB3 cells. (55pp)

8/7/19
DIALOG(R)File 357:Derwent Biotech Res.
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0236812 DBR Accession No.: 99-06913 PATENT
New autonomous parvo virus-based vector - recombinant virus vector-mediated

heterologous DNA transfer and expression in Escherichia coli for use in human cancer and infectious disease prevention and gene therapy
AUTHOR: Winocour E; Tal J; Corsini J A
CORPORATE SOURCE: Rehovot, Italy; Beer Sheva, Israel.
PATENT ASSIGNEE: Yeda-Res.Develop.; Univ.Negev-Ben-Gurion 1999
PATENT NUMBER: WO 9911802 PATENT DATE: 990311 WPI ACCESSION NO.: 99-205195 (9917)
PRIORITY APPLIC. NO.: IL 121676 APPLIC. DATE: 970901
NATIONAL APPLIC. NO.: WO 98IL419 APPLIC. DATE: 980828
LANGUAGE: English
ABSTRACT: An autonomous parvo virus-based vector (I) (minute virus of mice) which is capable of stably integrating a heterologous DNA into a desired specific target DNA, is new. The target DNA is a chromosomal or episomal site of a host cell, consisting of a DNA sequence containing binding and nicking sites recognized by an autonomous parvo virus regulatory protein for the initiation of localized DNA synthesis and recombination. The vector contained a binding site recognized by the regulatory protein, the replication origin (ori) of (I), a DNA sequence encoding the regulatory protein and a heterologous DNA to be integrated. Also claimed is a 53 bp oligonucleotide (II) (specified) which contains a sequence based on/related to the 3' or 5' ori and inserted into a host cell (e.g. Escherichia coli), becoming a target for (I) directed integration of a heterologous DNA and a method for identifying sites for site-specific chromosomal and episomal integration of the heterologous DNA into the host cell. The vector may be useful for the correction of a genetic defect, for the prevention or treatment of cancer, or infectious diseases in humans. (45pp)

8/7/27
DIALOG(R)File 357:Derwent Biotech Res.
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0221156 DBR Accession No.: 98-02753 PATENT
Mini-adenoviral vectors carrying the minimal cis-element of the adenoviral genome - adeno virus vector construction in packaging cell culture, and green fluorescent protein or human Factor-VIII expression in a transgenic mouse model for gene therapy testing
AUTHOR: Zhang W W; Alemany R; Dai Y; Josephs S; Balague C; Ayares D; Schneiderman R
CORPORATE SOURCE: Deerfield, IL, USA.
PATENT ASSIGNEE: Baxter 1997
PATENT NUMBER: WO 9745550 PATENT DATE: 971204 WPI ACCESSION NO.: 98-032656 (9803)
PRIORITY APPLIC. NO.: US 791218 APPLIC. DATE: 970131
NATIONAL APPLIC. NO.: WO 97US10218 APPLIC. DATE: 970530
LANGUAGE: English
ABSTRACT: A new DNA sequence has an adeno virus-5 (Ad5) inverted terminal repeat (ITR), a packaging signal, a transcription control region, an effector or reporter gene, and a genomic integration (e.g. a albumin or alpha-fetoprotein Alb-E5 AFP-3 or EBB14 gene homologous recombination arm, or an adeno-associated virus ITR) or episomal maintenance (e.g. a human or SV40 virus replication origin, alphoid DNA, T-antigen or oriP and Epstein-Barr virus nuclear antigen-1) sequence, where the remainder of the DNA does not encode an Ad protein. A human telomere, a human albumin or alpha-1-antitrypsin promoter and a liver-specific enhancer may also be present. The reporter gene may encode green fluorescent protein, and the effector gene may encode human Factor-VIII. The vector may be packaged in a cell culture to give an infectious replication-defective Ad vector. The vector may be used to generate a transgenic mouse by microinjection into embryonic stem cells. Plasmid pCMV-hFVIII-mini, AFP-pEGFP-1, mAFP-hFVIII/pGKNeo and RIP-pEGFP-from-BS vectors are new. The transgenic mouse may be used to test gene therapy strategies using the vectors. (193pp)

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13/KWIC/1
DIALOG(R)File 357:(c) 2003 Thomson Derwent & ISI. All rts. reserv.
...vector, by introducing vector having nucleotide sequence, adenovirus inverted terminal repeats and packaging sequence, and adeno - associated virus terminal repeat, into cell, and culturing cell - virus vector expression in host cell use...
...ABSTRACT: terminal repeats of adenovirus flanking NS, adenovirus packaging sequence linked to inverted terminal repeat, and adeno - associated virus terminal repeat linked to 3' end of NS, into cell expressing adenovirus early gene...
...flanking NS, adenovirus packaging sequence linked to one of the inverted terminal repeats, and an adeno - associated virus terminal repeat sequence operably linked to the 3' end of NS, where the first vector lacks a second adeno - associated virus terminal repeat sequence, and lacks one or more adenovirus early gene region such as...
... gene which is lacking from the first vector; and (iii) optionally a helper adenovirus or adeno - associated virus; (b) introducing the first vector, and optionally the genome of a helper adenovirus or adeno - associated virus, into the cell to produce a transformed cell; and (c) culturing the transformed cell...
...to produce a second vector chosen from: (i) a third vector comprising in operable combination adeno - associated virus terminal repeat DD sequence, first and second inverted copies of NS flanking the adeno - associated virus terminal repeat-DD sequence, left and right inverted terminal repeats of adenovirus flanking the...
... lacks adenovirus E3 early gene region, or by providing the first recombinant vector that comprises adeno - associated virus rep gene region, introducing first vector into the cell, and culturing the transformed cell...
... rep proteins and to produce a second vector. WIDER DISCLOSURE - (1) recombinant vectors including adenovirus/ adeno - associated virus vectors and mini-adenovirus (mAd) vectors; (2) cells containing the vectors of (1); and...
...as E1, E2, and E4 gene region, when the method involves the first vector comprising adeno - associated virus rep gene region. ACTIVITY - Immunostimulant; Antianemic; Antilipemic; Nootropic; Cytostatic; Dermatological. No biological data is...
... and dimeric mini-adenoviruses (mAd) in exemplary 293 cells were performed as follows. Recombinant adenovirus/ adeno - associated virus (Ad/ AAV) hybrid virus had the left end of Ad5 containing the inverted terminal repeat (ITR) and packaging domain, the AAV TR D sequence, an green fluorescent protein (EGFP)/neomycin (Neo) expression cassette from the plasmid pTRUF2, an intact AAV terminal repeat with a double D sequence (TR-DD), and the remainder of the Ad...
... from this virus backbone (E1 deletion). For generation of mini-adenoviruses using the parental Ad/ AAV EGFP/Neo virus, 293 cells which complement the E1 deletion in the hybrid virus to allow virus replication were infected with a cellular lysate containing the parental Ad/ AAV hybrid virus. Two days after infection, cleared cellular lysates were prepared and treated with DNase I and RNase A. Ad/ AAV and mAd viruses were separated on a CsCl2 step gradient. The lower band represented full...
... resistant, confirming that it was packaged within the virions. During normal replication of wild type AAV with an Ad helper virus, both monomer length as well as dimer length AAV genome products were observed as part of the replication pathway. DNA analysis was also carried out on the viral particles, which demonstrated that full virus particles contained the parental Ad/ AAV hybrid virus genome as a single DNA molecule 36 kbase in size. The E/M...
... DNA (nucleotides 1-420) containing the Ads ITR and packaging domain, as well as the AAV TR D sequence. The remainder of the AAV terminal repeat was missing from the mAd genome. The dimeric form contained a duplicated monomer genome where the left end of Ad5 (nucleotides 1-420), AAV TR D sequence and the EGFP-Neo expression cassette were duplicated in an inverted manner. An intact AAV TR was present at the junction of the duplication. (191 pages)
DESCRIPTORS: recombinant adeno virus, adeno - associated virus vector-mediated gene transfer expression in mouse, human host cell, inverted terminal repeat, packaging...
...familial hypercholesterolemia, Lesch-Nyhan syndrome, phenylalanine hydroxylas phenylketonuria, muscular dystrophy, cystic fibrosis therapy, gene therapy parvo virus mammal animal immunostimulant antianemic antipemic nootropic cytostatic (22, 07)
? s s12(3n)s10 and s1
16166 S12
148756 S10
317 S12(3N)S10
1542 S1
S14 3 S12(3N)S10 AND S1
? t s14/kwic/1-3
14/KWIC/1
DIALOG(R)File 357:(c) 2003 Thomson Derwent & ISI. All rts. reserv.
...ABSTRACT: at undetectable levels at day 1 after injection. By day 35, expression had increased by two logs and was 3 - 4 fold higher than the levels of CAT from pCFI-CAT at this time point...
... hybrid promoter is also useful in the context of viral vectors, e.g. adenovirus and adeno - associated vectors, and in other tissues such as muscle or brain. ADVANTAGE - (V) confers high and...

14/KWIC/2
DIALOG(R)File 357:(c) 2003 Thomson Derwent & ISI. All rts. reserv.

Sustained high-level expression of human factor IX (hFIX) after liver-targeted delivery of recombinant adeno - associated virus encoding the hFIX gene in rhesus macaques - vector-mediated gene transfer and expression in...
...ABSTRACT: using hyperimmune serum from a rhesus monkey that had received an adenoviral vector encoding hFIX. Two macaques having 3 and 40 rAAV genome equivalents/cell, respectively, in liver tissue had 4% and 8% of...
DESCRIPTORS: adeno - associated virus vector-mediated human Factor-IX, cytomegalo virus enhancer, beta-actin promoter gene transfer, expression...
...polymerasechain reaction, SDS-PAGE, ELISA, antibody, Western blot hybridization analysis, appl. hemophilia B gene therapy parvo virus mammal animal blood-clotting protein herpes virus embryo kidney DNA amplification analysis immunoassay DNA...

14/KWIC/3
DIALOG(R)File 357:(c) 2003 Thomson Derwent & ISI. All rts. reserv.
Mutational analysis of the adeno - associated virus type 2 (AAV2) capsid gene and construction of AAV2 vectors with altered tropism
ABSTRACT: To obtain a comprehensive genetic map of the adeno - associated virus capsid gene, 93 mutants at 59 different positions in the AAV capsid gene were constructed by site-directed mutagenesis. Several types of mutants were studied including...
... in what were likely to be beta-barrel in the capsid protein virus particle (VP)- 3 . Two of these mutants were insertions at the N and C termini of VP3, suggesting that...
... ligand in the N-terminal regions of VP1 or VP2 could change the tropism of AAV . The results provided information on AAV capsid functional domains and are useful for future design of AAV vectors for targeting of specific tissues. (51 ref)
DESCRIPTORS: adeno - associated virus vector construction, site-directed mutagenesis, capsid gene, altered tropism, appl. gene therapy parvo virus (Vol.19, No.22)
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S2 4872 TERMINUS OR TERMINI OR TELOMER?
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S6 55 S5 NOT S3
S7 1772 ORIGIN (2N)REPLICATION
S8 46 S1 AND S7
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$58.96 Estimated cost this search
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Logging in to Dialog
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File 357:Derwent Biotech Res. _1982-2003/Mar W2
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*File 357: File is now current. See HELP NEWS 357.
Alert feature enhanced for multiple files, etc. See HELP ALERT.

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File 155:MEDLINE(R) 1966-2003/Mar W1
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99279 MINIMAL
137442 ORIGIN
S2 80 MINIMAL (W)ORIGIN
? s s1 and s2
1704 S1
80 S2
S3 2 S1 AND S2
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3/7/1
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.
14198590 22326831 PMID: 12438604
A cis-acting element that directs circular adeno-associated virus replication and packaging.
Musatov Sergei; Roberts Jill; Pfaff Donald; Kaplitt Michael; et al
Laboratory of Neurobiology and Behavior, The Rockefeller University, Weill Medical College of Cornell University, 525 East 68th Street, New York, NY 10021, USA.
Journal of virology (United States) Dec 2002, 76 (24) p12792-802, ISSN 0022-538X Journal Code: 0113724
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
A novel pathway of adeno-associated virus (AAV) replication marked by the assembly of circular monomer duplex intermediates (cAAV) has been recently discovered. In the present report we identify a single AD domain of the inverted terminal repeat as a minimal origin of cAAV replication. A small internal palindrome (BB'), necessary for optimal Rep-inverted terminal repeat interaction, does not contribute to the efficiency of cAAV replication, while the terminal resolution site is an essential cis-acting element. Furthermore, recombinant cAAV vectors that encompass only the AD domain replicate exclusively in a circular form and no detectable linear duplex replicative intermediates are generated, suggesting that both pathways of AAV replication are independent and can be separated. In addition, we show that cAAVs are efficient templates for encapsidation of single-stranded DNA genomes, an observation that assigns a biological role for these novel replication species. Together, these findings shed new light on the current model of AAV replication and packaging.
Record Date Created: 20021119
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S4 7894 NICK
? s s1 and s4
1704 S1
7894 S4
S5 12 S1 AND S4
? t s5/7/7 9 10 11
5/7/7
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.
09385303 97296253 PMID: 9151837
The Rep78 gene product of adeno-associated virus (AAV) self-associates to form a hexameric complex in the presence of AAV ori sequences.
Smith R H; Spano A J; Kotin R M
Molecular Hematology Branch, National Heart, Lung, and Blood Institute, Bethesda, Maryland 20892, USA.
Journal of virology (UNITED STATES) Jun 1997, 71 (6) p4461-71, ISSN 0022-538X Journal Code: 0113724
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The Rep78 and Rep68 proteins of adeno-associated virus (AAV) are replication initiator proteins that bind the viral replicative-form origin of replication, nick the origin in a site- and strand-specific fashion, and mediate vectorial unwinding of the DNA duplex via an ATP-dependent helicase activity, thus initiating a strand displacement mechanism of viral DNA replication. Genetic and biochemical studies have identified Rep mutants that demonstrate a trans-dominant negative phenotype in vitro and in vivo, suggesting the possibility that multimerization of Rep is essential for certain replicative functions. In this study, we have investigated the ability of the largest of the Rep proteins, Rep78, to self-associate in vitro and in vivo. Self-association of Rep78 in vivo was demonstrated through the use of a mammalian two-hybrid system. Rep-Rep protein interaction was confirmed in vitro through coimmunoprecipitation experiments with a bacterially expressed maltose-binding protein-Rep78 fusion protein in combination with [35S]methionine-labeled Rep78 synthesized in a coupled in vitro transcription-translation system. Mapping studies with N- and C-terminal truncation mutant forms of Rep indicate that amino acid sequences required for maximal self-association occur between residues 164 and 484. Site-directed mutagenesis identified two essential motifs within this 321-amino-acid region: (i) a putative alpha-helix

bearing a 3,4-hydrophobic heptad repeat reminiscent of those found in coiled-coil domains and (ii) a previously recognized nucleoside triphosphate-binding motif. Deletion of either of these regions from the full-length polypeptide resulted in severe impairment of Rep-Rep interaction. In addition, gel filtration chromatography and protein cross-linking experiments indicated that Rep78 forms a hexameric complex in the presence of AAV ori sequences.
Record Date Created: 19970609

5/7/9
DIALOG(R)File 155:MEDLINE(R)

08261289 95018658 PMID: 7933128
Sequence requirements for stable binding and function of Rep68 on the adeno-associated virus type 2 inverted terminal repeats.
Chiorini J A; Wiener S M; Owens R A; Kyostio S R; Kotin R M; Safer B
Molecular Hematology Branch, National Heart, Lung, and Blood Institute, Bethesda, MD 20892-1654.
Journal of virology (UNITED STATES) Nov 1994, 68 (11) p7448-57, ISSN 0022-538X Journal Code: 0113724
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Replication of the palindromic inverted terminal repeats (ITRs) of adeno-associated virus type 2 requires several functions of the viral nonstructural Rep proteins. These include binding to the ITR, nicking of the double-stranded replication intermediate at the terminal resolution site (trs), and then strand displacement and synthesis from the nick. This report demonstrates the ability of both recombinant fusion maltose-binding protein (MBP)-Rep68 delta produced in Escherichia coli and wild-type (wt) Rep68 to bind to a linear truncated form of the ITR, delta 57 ITR, with similar affinity as to the wt hairpin ITR. A dissociation constant for MBP-Rep68 delta of approximately 8 x 10(-10) M was determined for the wt ITR and delta 57 ITR probes. Truncation of delta 57 ITR to generate delta 28 ITR, which retains the GCTC repeat motif but not the trs, bound at least 10 times less efficiently than delta 57 ITR. Extension of delta 28 ITR with nonspecific sequence restored the ability of MBP-Rep68 delta to bind to delta 28 ITR. Thus, high-affinity binding would appear to require stabilization by flanking sequence as well as the intact GCTC repeat motif. Cleavage of the delta 57 ITR probe with DdeI, which truncates the flanking sequence and was previously shown to inhibit binding by Rep68, also inhibited the binding of MBP-Rep68 delta. The requirements for stable binding were further defined with a series of oligonucleotide probes which spanned the region protected by MBP-Rep78 in DNase I footprinting. The binding activity of either MBP-Rep68 delta or wt Rep68 to hairpin ITR or delta 57 ITR was indistinguishable. However, the binding activity of MBP-Rep68 delta to DNA does not appear to correlate with trs endonuclease activity. The nicking and covalent linkage of MBP-Rep68 delta to the nonhairpin delta 57 ITR was approximately 100-fold less efficient than its linkage to a hairpin-containing ITR. Therefore, although the hairpin portion of the ITR does not appear to play a role in recognition and stabilization of MBP-Rep68 delta binding, its presence does affect the trs cleavage activity of the protein.
Record Date Created: 19941117

5/7/10
DIALOG(R)File 155:MEDLINE(R)

08200448 94335120 PMID: 8057478
Adeno-associated virus DNA replication in vitro: activation by a maltose binding protein/Rep 68 fusion protein.
Ward P; Urcelay E; Kotin R; Safer B; Berns K I
Department of Microbiology, Hearst Microbiology Research Center, Cornell University Medical College, New York, New York 10021.
Journal of virology (UNITED STATES) Sep 1994, 68 (9) p6029-37, ISSN 0022-538X Journal Code: 0113724
Contract/Grant No.: GM 50032; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The adeno-associated virus (AAV) nonstructural protein Rep 68 is required for viral DNA replication. An in vitro assay has been developed in which addition of Rep 68 to an extract from uninfected HeLa cells supports AAV DNA replication. In this paper, we report characterization of the replication process when a fusion of the maltose binding protein and Rep 68, expressed in Escherichia coli, was used in the assay. Replication was observed when the template was either linear double-stranded AAV DNA or a plasmid construct containing intact AAV DNA. When the recombinant plasmid construct was used as the template, there was replication of pBR322 DNA as well as the AAV DNA; however, linear pBR322 DNA was not replicated. When the plasmid construct was the template, replication appeared to initiate on the intact plasmid and led to separation of the AAV sequences from those of the vector, a process which has been termed rescue. There was no evidence that replication could initiate on the products of rescue. Rep 68 can make a site-specific nick 124 nucleotides from the 3' end of AAV DNA; the site of the nick has been called the terminal resolution site. Our data are most consistent with initiation occurring at the terminal resolution site and proceeding toward the 3' terminus. When the template was the plasmid construct, either elongation continued past the junction into pBR322 sequences or the newly synthesized sequence hairpinned, switched template strands, and replicated the AAV DNA. Replication was linear for 4 h, during which time 70% of the maximal synthesis took place. An additional finding

was that the Rep fusion could resolve AAV dimer length duplex intermediates into monomer duplexes without DNA synthesis.
Record Date Created: 19940909

5/7/11
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.
07850201 93381812 PMID: 8396670
Features of the adeno-associated virus origin involved in substrate recognition by the viral Rep protein.
Snyder R O; Im D S; Ni T; Xiao X; Samulski R J; Muzyczka N
Department of Microbiology, State University of New York at Stony Brook Medical School 11794-8621.
Journal of virology (UNITED STATES) Oct 1993, 67 (10) p6096-104, ISSN 0022-538X Journal Code: 0113724
Contract/Grant No.: 5 PO1 CA2814607; CA; NCI; AI25530; AI; NIAID; RO1 GM3572302; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We previously demonstrated that the adeno-associated virus (AAV) Rep68 and Rep78 proteins are able to nick the AAV origin of DNA replication at the terminal resolution site (trs) in an ATP-dependent manner. Using four types of modified or mutant substrates, we now have investigated the substrate requirements of Rep68 in the trs endonuclease reaction. In the first kind of substrate, portions of the hairpinned AAV terminal repeat were deleted. Only deletions that retained virtually all of the small internal palindromes of the AAV terminal repeat were active in the endonuclease reaction. This result confirmed previous genetic and biochemical evidence that the secondary structure of the terminal repeat was an important feature for substrate recognition. In the second type of substrate, the trs was moved eight bases further away from the end of the genome. The mutant was nicked at a 50-fold-lower frequency relative to a wild-type origin, and the nick occurred at the correct trs sequence despite its new position. This finding indicated that the endonuclease reaction required a specific sequence at the trs in addition to the correct secondary structure. It also suggested that the minimum trs recognition sequence extended three bases from the cut site in the 3' direction. The third type of substrate harbored mismatched base pairs at the trs. The mismatch substrates contained a wild-type sequence on the strand normally cut but an incorrect sequence on the complementary strand. All of the mismatch mutants were capable of being nicked in the presence of ATP. However, there was substantial variation in the level of activity, suggesting that the sequence on the opposite strand may also be recognized during nicking. Analysis of the mismatch mutants also suggested that a single-stranded trs was a viable substrate for the enzyme. This interpretation was confirmed by analysis of the fourth type of substrate tested, which contained a single-stranded trs. This substrate was also cleaved efficiently by the enzyme provided that the correct strand was present in the substrate. In addition, the single-stranded substrate no longer required ATP as a cofactor for nicking. Finally, all of the substrates with mutant trss bound the Rep protein as efficiently as the wild-type did. This finding indicated that the sequence at the cut site was not involved in recognition of the terminal repeat for specific binding by the enzyme. We concluded that substrate recognition by the AAV Rep protein involves at least two and possibly as many as four features of the AAV terminal repeat.(ABSTRACT TRUNCATED AT 400 WORDS)
Record Date Created: 19931012
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$1.05 5 Type(s) in Format 7
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File 155:MEDLINE(R) 1966-2003/Mar W1
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S3 2 S1 AND S2
S4 7894 NICK

S5 12 S1 AND S4
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137442 ORIGIN
96349 REPLICATION
S6 1829 ORIGIN (1W) REPLICATION
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1704 S1
1829 S6
S7 12 S1 AND S6
? t s7/7/12
7/7/12
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.
06924727 91237836 PMID: 2033669
Origin of adeno-associated virus DNA replication is a target of carcinogen-inducible DNA amplification.
Yalkinoglu A O; Zentgraf H; Hubscher U
Institut fur Virusforschung/Angewandte Tumorvirologie, Deutsches Krebsforschungszentrum, Heidelberg, Federal Republic of Germany.
Journal of virology (UNITED STATES) Jun 1991, 65 (6) p3175-84, ISSN 0022-538X Journal Code: 0113724
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
DNA amplification of the helper-dependent parvovirus AAV (adeno-associated virus) can be induced by a variety of genotoxic agents in the absence of coinfecting helper virus. Here we investigated whether the origin of AAV type 2 DNA replication cloned into a plasmid is sufficient to promote replication activity in cells treated by the carcinogen N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). A pUC19-based plasmid, designated pA2Y1, which contains the left terminal repeat sequences (TRs) representing the AAV origin of replication and the p5 and p19 promoter but lacks any functional parvoviral genes is shown to confer replication activity and to allow selective DNA amplification in carcinogen-treated cells. Following transfection of plasmid pA2Y1 or plasmid pUC19 as a control, density labeling by a bromodeoxyuridine and DpnI resistance assay suggested a semi-conservative mode of replication of the AAV origin-containing plasmid. Furthermore, the amount of DpnI-resistant full-length pA2Y1 DNA molecules was increased by MNNG treatment of cells in a dose-dependent manner. In addition, DNA synthesis of plasmid pA2Y1 was studied in vitro. Extracts derived from MNNG-treated CHO-9 and L1210 cells displayed greater synthesis of DpnI-resistant full-length pA2Y1 molecules than did nontreated controls. Experiments with specific enzyme inhibitors suggested that the reaction is largely dependent on DNA polymerase alpha, DNA primase, and DNA topoisomerase I. Furthermore, restriction endonuclease mapping analysis of the in vitro reaction products revealed the occurrence of specific initiation at the AAV origin of DNA replication. Though elongation was not very extensive, extracts from carcinogen-treated cells markedly amplified the AAV origin region. Our results, including electron microscopic examination, suggest that the AAV origin/terminal repeat structure is recognized by the cellular DNA replicative machinery induced or modulated by carcinogen treatment in the absence of parvoviral gene products.
Record Date Created: 19910626
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DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.
12699923 21574575 PMID: 11707592
Adeno-associated virus (AAV) site-specific recombination does not require a Rep-dependent origin of replication within the AAV terminal repeat.
Young S M; Samulski R J
Curriculum in Genetics and Molecular Biology, Gene Therapy Center, University of North Carolina, Chapel Hill, NC 27599, USA.
Proceedings of the National Academy of Sciences of the United States of America (United States) Nov 20 2001, 98 (24) p13525-30, ISSN 0027-8424 Journal Code: 7505876
Contract/Grant No.: DK51880; DK; NIDDK; HL 48347; HL; NHLBI
Document type: Journal Article
Languages: ENGLISH
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Adeno-associated virus (AAV) is the only known eukaryotic virus capable of targeted integration in human cells. An AAV Rep binding element (RBE) and terminal resolution site (trs) identical to the viral terminal repeats required for AAV DNA replication are located on chromosome (ch) 19. Both ch-19 RBE and trs elements have been shown to be essential for viral targeting to this locus. To characterize the role of the AAV inverted terminal repeat (ITR) cis-acting sequences in targeted integration an AAV trs mutant incapable of supporting viral replication was tested. Wild-type and mutant substrates were assayed for targeted integration after cotransfection in the presence or absence of Rep. Our results demonstrated that, in the presence of Rep78, both ITR substrates targeted to ch-19 with similar frequency. Molecular characterization of the mutant ITR integrants confirmed the presence of the trs mutation in the majority of samples tested. Complementation analysis confirmed that the mutant targeted viral genomes were unable to rescue and replicate. In addition, Rep78 induced extensive rearrangement and amplification of ch-19 sequences independent of wild-type or mutant targeting substrate. These studies demonstrate that Rep-dependent nicking of the viral cis-acting trs sequence is not a prerequisite for site-specific recombination and suggests AAV targeting is mediated by Rep78/68-dependent replication from the ch-19 origin of replication (ori). These studies have significant impact toward the

understanding of AAV site-specific recombination and the development of targeting vectors.
Record Date Created: 20011121

7/7/11
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.
07760914 93287272 PMID: 8389942
Analysis of the terminal repeat binding abilities of mutant adeno-associated virus replication proteins.
Yang Q; Trempe J P
Department of Biochemistry and Molecular Biology, Medical College of Ohio, Toledo 43699-0008.
Journal of virology (UNITED STATES) Jul 1993, 67 (7) p4442-7, ISSN 0022-538X Journal Code: 0113724
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The adeno-associated virus (AAV) Rep78 and Rep68 proteins play essential roles in viral DNA replication, trans activation of viral gene expression, and suppression of oncogene-mediated cellular transformation. By using an extensive set of linker insertion and deletion mutations in the replication gene, we mapped the regions of the Rep78 protein that mediate binding to the AAV origin of replication in vitro. Deletions that removed amino acid codons 25 to 62, 88 to 113, 125 to 256, and 346 to 400 abolished binding. Alterations in several other regions of the protein affected the binding affinity of the mutant proteins. All of the mutant proteins that support AAV DNA replication or p40 trans activation bound to the terminal repeat sequence, thus verifying the importance of binding for these functions. Several mutant rep genes that failed to suppress oncogene-mediated cellular transformation produced proteins that were capable of binding to the AAV terminal repeat sequences.
Record Date Created: 19930712
? s trs or terminal(w)resolution(w)(site or sites)
769 TRS
191235 TERMINAL
88924 RESOLUTION
336559 SITE
364178 SITES
33 TERMINAL(W)RESOLUTION(W)(SITE OR SITES)
S8 781 TRS OR TERMINAL(W)RESOLUTION(W)(SITE OR SITES)
? ds
Set Items Description
S1 1704 AAV OR ADENOASSOCIAT? OR ADENO(W)ASSOCIAT?
S2 80 MINIMAL (W)ORIGIN
S3 2 S1 AND S2
S4 7894 NICK
S5 12 S1 AND S4
S6 1829 ORIGIN (1W) REPLICATION
S7 12 S1 AND S6
S8 781 TRS OR TERMINAL(W)RESOLUTION(W)(SITE OR SITES)
? s s1 and s8
1704 S1
781 S8
S9 39 S1 AND S8
? t s9/7/23
9/7/23
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.
08538652 95297175 PMID: 7778304
Minimum origin requirements for linear duplex AAV DNA replication in vitro.
Ward P; Berns K I
Department of Microbiology, W. R. Hearst Microbiology Research Center, Cornell University Medical College, New York, New York 10021, USA.
Virology (UNITED STATES) Jun 1 1995, 209 (2) p692-5, ISSN 0042-6822 Journal Code: 0110674
Contract/Grant No.: GM50023; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We have investigated the minimal requirements for a functional origin of DNA replication in an in vitro assay which requires the adeno-associated virus (AAV) Rep 68/78 protein. When a linear duplex template was used, initiation of one round of DNA replication was achieved when nucleotides 1-56 of the AAV inverted terminal repeat (in the flop orientation) were present at the terminus of the template. A terminal resolution site was not required. Deletion of an additional 11 nucleotides from the terminal repeat blocked the reaction.
Record Date Created: 19950711
? t s9/7/10 18 21 25 27 28 30 31
9/7/10
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.
10226729 99214370 PMID: 10196327
Adeno-associated virus (AAV) type 5 Rep protein cleaves a unique terminal resolution site compared with other AAV serotypes.
Chiorini J A; Afione S; Kotin R M
Molecular Hematology Branch, National Heart, Lung, and Blood Institute, Bethesda, Maryland 20892, USA.
Journal of virology (UNITED STATES) May 1999, 73 (5) p4293-8, ISSN 0022-538X Journal Code: 0113724

Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Adeno-associated virus (AAV) replication depends on two viral components for replication: the AAV nonstructural proteins (Rep) in trans, and inverted terminal repeat (ITR) sequences in cis. AAV type 5 (AAV5) is a distinct virus compared to the other cloned AAV serotypes. Whereas the Rep proteins and ITRs of other serotypes are interchangeable and can be used to produce recombinant viral particles of a different serotype, AAV5 Rep proteins cannot cross-complement in the packaging of a genome with an AAV2 ITR. In vitro replication assays indicated that the block occurs at the level of replication instead of at viral assembly. AAV2 and AAV5 Rep binding activities demonstrate similar affinities for either an AAV2 or AAV5 ITR; however, comparison of terminal resolution site (TRS) endonuclease activities showed a difference in specificity for the two DNA sequences. AAV2 Rep78 cleaved only a type 2 ITR DNA sequence, and AAV5 Rep78 cleaved only a type 5 probe efficiently. Mapping of the AAV5 ITR TRS identified a distinct cleavage site (AGTG TGGC) which is absent from the ITRs of other AAV serotypes. Comparison of the TRSs in the AAV2 ITR, the AAV5 ITR, and the AAV chromosome 19 integration locus identified some conserved nucleotides downstream of the cleavage site but little homology upstream.
Record Date Created: 19990519

9/7/18
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.
09268828 97151099 PMID: 8995635
A novel terminal resolution-like site in the adeno-associated virus type 2 genome.
Wang X S; Srivastava A
Department of Medicine, Walther Oncology Center, Indiana University School of Medicine, Indianapolis 46202, USA.
Journal of virology (UNITED STATES) Feb 1997, 71 (2) p1140-6, ISSN 0022-538X Journal Code: 0113724
Contract/Grant No.: DK-49218; DK; NIDDK; HL-48342; HL; NHLBI; HL-53586; HL; NHLBI
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The adeno-associated virus 2 (AAV) contains a single-stranded DNA genome of which the terminal 145 nucleotides are palindromic and form T-shaped hairpin structures. These inverted terminal repeats (ITRs) play an important role in AAV DNA replication and resolution, since each of the ITRs contains a terminal resolution site (trs) that is the target site for the AAV rep gene products (Rep). However, the Rep proteins also interact with the AAV DNA sequences that lie outside the ITRs, and the ITRs also play a crucial role in excision of the proviral genome from latently infected cells or from recombinant AAV plasmids. To distinguish between Rep-mediated excision of the viral genome during rescue from recombinant AAV plasmids and the Rep-mediated resolution of the ITRs during AAV DNA replication, we constructed recombinant AAV genomes that lacked either the left or the right ITR sequence and one of the Rep-binding sites (RBSs). No rescue and replication of the AAV genome occurred from these plasmids following transfection into adenovirus type 2-infected human KB cells, as expected. However, excision and abundant replication of the vector sequences was clearly detected from the plasmid that lacked the AAV left ITR, suggesting the existence of an additional putative excision site in the left end of the AAV genome. This site was precisely mapped to one of the AAV promoters at map unit 5 (AAV p5) that also contains an RBS. Furthermore, deletion of this RBS abolished the rescue and replication of the vector sequences. These studies suggest that the Rep-mediated cleavage at the RBS during viral DNA replication may, in part, account for the generation of the AAV defective interfering particles.
Record Date Created: 19970218

9/7/21
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.
08851234 96190551 PMID: 8627673
Sequence requirements for binding of Rep68 to the adeno-associated virus terminal repeats.
Ryan J H; Zolotukhin S; Muzyczka N
Department of Molecular Genetics and Microbiology, College of Medicine, University of Florida, Gainesville 32610, USA.
Journal of virology (UNITED STATES) Mar 1996, 70 (3) p1542-53, ISSN 0022-538X Journal Code: 0113724
Contract/Grant No.: HL/DK 50257; HL; NHLBI; PO1 CA2814607; CA; NCI; RO1 GM3572302; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
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We have used reciprocal competition binding experiments with mutant substrates and chemical modification interference assays to precisely define the sequences within the adeno-associated virus (AAV) terminal repeat (TR) that are involved in site-specific binding to the AAV Rep protein. Mutagenesis experiments were done with a 43-bp oligonucleotide which contained the Rep binding element (RBE) within the A stem of the TR. Experiments in which two adjacent base pairs of the RBE were substituted simultaneously with nucleotides that produced transversions identified a 22-bp sequence (CAGTGAGCGAGCGAGCGCGCAG) in which substitutions

measurably
affected the binding affinity. Although the 22-bp RBE contains the GAGC motifs that have been found in all known Rep binding sites, our results suggest that the GAGC motifs alone are not the only sequences specifically recognized by Rep. The effects of substitutions within the 22-bp sequence were relatively symmetrical, with nucleotides at the periphery of the RBE having the least effect on binding affinity and those in the middle having the greatest effect. Dinucleotide mutations within 18 (GTGAGCGAGCGAGC) of the 22 bp were found to decrease the binding affinity by at least threefold. Dinucleotide mutations within a 10-bp core sequence (GCGAGCGAGC) were found to decrease binding affinity by more than 10-fold. Single-base substitutions within the 10-bp core sequence lowered the binding affinity by variable amounts (up to fivefold). The results of the mutagenesis analysis suggested that the A-stem RBE contains only a single Rep binding site rather than two or more independent sites. To confirm the results of the mutant analysis and to determine the relative contribution of each base to binding, chemical modification experiments using dimethyl sulfate and hydrazine were performed on both the linear A-stem sequence and the entire AAV TR in both the flip and flop hairpinned configurations. Interference assays on the linear A stem identified the 18-bp sequence described above as essential for binding. G, C, and T residues on both strands contributed to binding, and the interference pattern correlated well with the results of the mutagenesis experiments. Interference assays with complete hairpinned TR substrates also identified the 18-bp sequence as important for binding. However, the interference patterns on the two strands within the RBE and the relative contributions of the individual bases to binding were clearly different between the hairpinned substrates and the linear A-stem binding element. Interference assays also allowed us to search for residues within the small internal palindromes of the TR (B and C) that contribute to binding. The largest effect was seen by modification of two T residues within the sequence CTTTG. This sequence was present in the same position relative to the terminal resolution site (trs) in both the flip and flop orientations of the TR. In addition, the interference pattern suggested that the remaining bases within the CTTTG motif as well as other bases within the B and C palindromes make contacts with the Rep protein, albeit with lower affinities. Regardless of whether the TR was in the flip or flop orientation, most of the contact points were clustered in the small internal palindrome furthest away from the trs. We also determined the relative binding affinity of linear substrates containing a complete RBE with hairpinned substrates and found that linear substrates bound Rep less efficiently. Our results were consistent with our previous model that there are three distinct elements within the hairpinned AAV TR that contribute to binding affinity or to efficient nicking at the trs: the A-stem RBE, the secondary structure element which consists of the B and C palindromes, and the trs.
Record Date Created: 19960627

9/7/25
DIALOG(R)File 155:MEDLINE(R)

08261289 95018658 PMID: 7933128
Sequence requirements for stable binding and function of Rep68 on the adeno-associated virus type 2 inverted terminal repeats.
Chiorini J A; Wiener S M; Owens R A; Kyostio S R; Kotin R M; Safer B
Molecular Hematology Branch, National Heart, Lung, and Blood Institute, Bethesda, MD 20892-1654.
Journal of virology (UNITED STATES) Nov 1994, 68 (11) p7448-57, ISSN 0022-538X Journal Code: 0113724
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Replication of the palindromic inverted terminal repeats (ITRs) of adeno-associated virus type 2 requires several functions of the viral nonstructural Rep proteins. These include binding to the ITR, nicking of the double-stranded replication intermediate at the terminal resolution site (trs), and then strand displacement and synthesis from the nick. This report demonstrates the ability of both recombinant fusion maltose-binding protein (MBP)-Rep68 delta produced in Escherichia coli and wild-type (wt) Rep68 to bind to a linear truncated form of the ITR, delta 57 ITR, with similar affinity as to the wt hairpin ITR. A dissociation constant for MBP-Rep68 delta of approximately 8 x 10(-10) M was determined for the wt ITR and delta 57 ITR probes. Truncation of delta 57 ITR to generate delta 28 ITR, which retains the GCTC repeat motif but not the trs, bound at least 10 times less efficiently than delta 57 ITR. Extension of delta 28 ITR with nonspecific sequence restored the ability of MBP-Rep68 delta to bind to delta 28 ITR. Thus, high-affinity binding would appear to require stabilization by flanking sequence as well as the intact GCTC repeat motif. Cleavage of the delta 57 ITR probe with DdeI, which truncates the flanking sequence and was previously shown to inhibit binding by Rep68, also inhibited the binding of MBP-Rep68 delta. The requirements for stable binding were further defined with a series of oligonucleotide probes which spanned the region protected by MBP-Rep78 in DNase I footprinting. The binding activity of either MBP-Rep68 delta or wt Rep68 to hairpin ITR or delta 57 ITR was indistinguishable. However, the binding activity of MBP-Rep68 delta to DNA does not appear to correlate with trs endonuclease activity. The nicking and covalent linkage of MBP-Rep68 delta to the nonhairpin delta 57 ITR was approximately 100-fold less efficient than its linkage to a hairpin-containing ITR. Therefore, although the hairpin portion of the ITR does not appear to play a role in recognition and stabilization of MBP-Rep68 delta binding, its presence does affect the trs cleavage activity of the protein.
Record Date Created: 19941117

9/7/27
DIALOG(R)File 155:MEDLINE(R)

08174625 94309164 PMID: 8035499
Interaction of the adeno-associated virus Rep protein with a sequence within the A palindrome of the viral terminal repeat.
McCarty D M; Ryan J H; Zolotukhin S; Zhou X; Muzyczka N
Department of Microbiology, School of Medicine, University at Stony Brook, New York 11794.
Journal of virology (UNITED STATES) Aug 1994, 68 (8) p4998-5006, ISSN 0022-538X Journal Code: 0113724
Contract/Grant No.: PO1 CA2814607; CA; NCI; RO1 GM3572302; GM; NIGMS; T32 AI25530; AI; NIAID
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We have characterized a Rep binding sequence which is within the A stem region of the adeno-associated virus terminal repeat (TR) and compared its affinity with that of the complete hairpinned TR for pure Rep68. Both the A stem and the complete TR substrates produced a complex pattern of protein-DNA complexes in which at least six different bound species could be distinguished. Competition experiments suggested that the dissociation constant for the A stem sequence is approximately 125-fold higher than that for the complete TR. The competition experiments also suggested that the average number of Rep molecules per TR substrate molecule under conditions of saturating substrate is 3.7:1, while for the A stem substrate, the ratio is 10:1. In spite of the apparent difference in protein-to-DNA ratio in the complexes, no major difference was seen in the mobility or the pattern of the protein-DNA complexes with the two kinds of substrates, suggesting that the difference in protein-to-DNA ratio was due to the lower stability of the A stem complex rather than the actual number of Rep molecules per DNA molecule. At least some of the difference in stability of the two kinds of complexes was due to the fact that the dissociation rate of the A stem substrate from the protein-DNA complexes was approximately fourfold faster than that of the complete TR. The dissociation rate curves for both substrates, however, were complex, suggesting that substrate was being released from at least two different kinds of protein-DNA complexes at different rates. In addition, we have analyzed binding to several substitution mutants within the A stem of the TR. A five-base mutant near the terminal resolution site (trs site) had little effect on binding. Two other mutants produced seven- or five-base substitutions within the 25-bp sequence of the A stem that had been identified in the accompanying report (D. M. McCarty, D. J. Pereira, I. Zolotukhin, X. Zhou, J. H. Ryan, and N. Muzyczka, J. Virol. 68:4988-4997, 1994) as essential for binding. Each of these mutants eliminated some but not all of the repeating GAGC motifs in the 25-bp A stem region. Both of these mutants completely abolished binding to the A stem substrate but only partially reduced binding in the context of the complete hairpinned TR. Furthermore, neither mutant altered the pattern of Rep-DNA complexes produced.(ABSTRACT TRUNCATED AT 400 WORDS)
Record Date Created: 19940815

9/7/28
DIALOG(R)File 155:MEDLINE(R)

08174624 94309163 PMID: 8035498
Identification of linear DNA sequences that specifically bind the adeno-associated virus Rep protein.
McCarty D M; Pereira D J; Zolotukhin I; Zhou X; Ryan J H; Muzyczka N
Department of Microbiology, School of Medicine, University at Stony Brook, New York 11794.
Journal of virology (UNITED STATES) Aug 1994, 68 (8) p4988-97, ISSN 0022-538X Journal Code: 0113724
Contract/Grant No.: PO1 CA2814607; CA; NCI; RO1 GM3572302; GM; NIGMS; T32 AI25530; AI; NIAID
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
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We have used baculovirus-expressed Rep68 that has been purified to homogeneity to reexamine the binding properties of the Rep protein. We find that Rep68 is capable of binding to a linear DNA sequence that is contained within a 25-bp sequence of the A stem of the adeno-associated virus (AAV) terminal repeat proximal to the B and C palindromes. This has been shown conclusively by demonstrating that Rep68 could specifically bind to a synthetic oligonucleotide containing the 25-bp region in the absence of the other sequences within the terminal repeat. Rep78 was also capable of binding the A stem recognition element, as demonstrated by the fact that a DNA affinity column containing the 25-bp sequence can be used to purify Rep78. The ability to recognize the linear DNA sequence within the A stem provides a mechanism by which the Rep protein can be oriented on the terminal repeat so that only the correct strand is cut at the terminal resolution site (trs site) during terminal resolution. In addition, computer analysis suggests that sequences similar to the A stem element are present within the three AAV promoter regions. Electrophoretic mobility shift experiments clearly demonstrate that the p5 promoter contains a Rep binding sequence. DNase protection experiments indicate that the Rep binding sequence within the p5 promoter is located between the YY1 initiator sequence and the TATA binding site. This position immediately suggests a mechanism by which the Rep protein could act as a repressor or a transactivator of p5 transcription by interacting with either YY1 or TBP. In addition, gel shift experiments suggest that the p19 promoter also contains a Rep binding site. The presence of Rep binding sites upstream of

both promoters suggests that these sites may be involved in coordinate regulation of AAV transcription. In addition, we have identified a heterologous Rep binding sequence within pBR322 DNA. A comparison of the sequences within the A stem, p5, and pBR322 binding sites suggests that a repeating GAGC motif is at least part of the Rep recognition sequence. In the accompanying report (D. M. McCarty, J. H. Ryan, S. Zolutukhin, X. Zhou, and N. Muzyczka, J. Virol. 68:4998-5006, 1994), we examine the relative affinity of Rep to the A stem site and the complete terminal repeat. Finally, we also have reexamined the ability of Rep68 and Rep78 to cut at the trs site in substrates that do not contain the B and C palindromes or any apparent secondary structure.(ABSTRACT TRUNCATED AT 400 WORDS)
Record Date Created: 19940815

9/7/30
DIALOG(R)File 155:MEDLINE(R)

08001024 94118379 PMID: 8289342
In vitro replication of adeno-associated virus DNA.
Ni T H; Zhou X; McCarty D M; Zolotukhin I; Muzyczka N
Department of Microbiology, State University of New York, Stony Brook Medical School 11794.
Journal of virology (UNITED STATES) Feb 1994, 68 (2) p1128-38, ISSN 0022-538X Journal Code: 0113724
Contract/Grant No.: 5 PO1 CA2814607; CA; NCI; AI25530; AI; NIAID; RO1 GM3572302; GM; NIGMS; +
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The study of eukaryotic viral DNA replication in vitro has led to the identification of cellular enzymes involved in DNA replication. Adeno-associated virus (AAV) is distinct from previously reported systems in that it is believed to replicate entirely by leading-strand DNA synthesis and requires coinfection with adenovirus to establish completely permissive replication. In previous work, we demonstrated that two of the AAV nonstructural proteins, Rep78 and -68, are site-specific endonucleases and DNA helicases that are capable of resolving covalently closed AAV termini, a key step in AAV DNA replication. We have now cloned the AAV nonstructural proteins Rep78, Rep68, and Rep52 in the baculovirus expression system. Using the baculovirus-expressed proteins, we have developed an efficient in vitro AAV DNA replication system which mimics the in vivo behavior of AAV in every respect. With no-end AAV DNA as the starting substrate, the reaction required an adenovirus-infected cell extract and the presence of either Rep78 or Rep68. Rep52, as expected, did not support DNA replication. A mutant in the AAV terminal resolution site (trs) was defective for DNA replication in the in vitro assay. Little, if any, product was formed in the absence of the adenovirus-infected HeLa cell extract. In general, uninfected HeLa extracts were less efficient in supporting AAV DNA replication than adenovirus-infected extracts. Thus, the requirement for adenovirus infection in vivo was partially duplicated in vitro. The reduced ability of uninfected HeLa extracts to support complete DNA replication was not due to a defect in terminal resolution but rather to a defect in the reinitiation reaction or in elongation. Rep78 produced a characteristic monomer-dimer pattern of replicative intermediates, but surprisingly, Rep68 produced little, if any, dimer replicative form. The reaction had a significant lag (30 min) before incorporation of 32P-deoxynucleoside triphosphate could be detected in DpnI-resistant monomer replicative form and was linear for at least 4 h after the lag. The rate of incorporation in the reaction was comparable to that in the simian virus 40 in vitro system. Replication of the complete AAV DNA molecule was demonstrated by the following criteria. (i) Most of the monomer and dimer product DNAs were completely resistant to digestion with DpnI. (ii) Virtually all of the starting substrate was converted to heavy-light or heavy-heavy product DNA in the presence of bromo-dUTP when examined on CsCl density gradients.(ABSTRACT TRUNCATED AT 400 WORDS)
Record Date Created: 19940218

9/7/31
DIALOG(R)File 155:MEDLINE(R)

07850201 93381812 PMID: 8396670
Features of the adeno-associated virus origin involved in substrate recognition by the viral Rep protein.
Snyder R O; Im D S; Ni T; Xiao X; Samulski R J; Muzyczka N
Department of Microbiology, State University of New York at Stony Brook Medical School 11794-8621.
Journal of virology (UNITED STATES) Oct 1993, 67 (10) p6096-104, ISSN 0022-538X Journal Code: 0113724
Contract/Grant No.: 5 PO1 CA2814607; CA; NCI; AI25530; AI; NIAID; RO1 GM3572302; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We previously demonstrated that the adeno-associated virus (AAV) Rep68 and Rep78 proteins are able to nick the AAV origin of DNA replication at the terminal resolution site (trs) in an ATP-dependent manner. Using four types of modified or mutant substrates, we now have investigated the substrate requirements of Rep68 in the trs endonuclease reaction. In the first kind of substrate, portions of the hairpinned AAV terminal repeat were deleted. Only deletions that retained virtually all of the small internal palindromes of the AAV terminal repeat were active in the endonuclease reaction. This result confirmed previous genetic and biochemical evidence that the secondary structure of the terminal repeat

was an important feature for substrate recognition. In the second type of substrate, the trs was moved eight bases further away from the end of the genome. The mutant was nicked at a 50-fold-lower frequency relative to a wild-type origin, and the nick occurred at the correct trs sequence despite its new position. This finding indicated that the endonuclease reaction required a specific sequence at the trs in addition to the correct secondary structure. It also suggested that the minimum trs recognition sequence extended three bases from the cut site in the 3' direction. The third type of substrate harbored mismatched base pairs at the trs. The mismatch substrates contained a wild-type sequence on the strand normally cut but an incorrect sequence on the complementary strand. All of the mismatch mutants were capable of being nicked in the presence of ATP. However, there was substantial variation in the level of activity, suggesting that the sequence on the opposite strand may also be recognized during nicking. Analysis of the mismatch mutants also suggested that a single-stranded trs was a viable substrate for the enzyme. This interpretation was confirmed by analysis of the fourth type of substrate tested, which contained a single-stranded trs. This substrate was also cleaved efficiently by the enzyme provided that the correct strand was present in the substrate. In addition, the single-stranded substrate no longer required ATP as a cofactor for nicking. Finally, all of the substrates with mutant trss bound the Rep protein as efficiently as the wild-type did. This finding indicated that the sequence at the cut site was not involved in recognition of the terminal repeat for specific binding by the enzyme. We concluded that substrate recognition by the AAV Rep protein involves at least two and possibly as many as four features of the AAV terminal repeat.(ABSTRACT TRUNCATED AT 400 WORDS)

Record Date Created: 19931012

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$9.11 Estimated cost this search

$9.11 Estimated total session cost 1.258 DialUnits

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